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(54) Title: IMPROVED FLOW CYTOMETRY APPARATUS AND METHOD
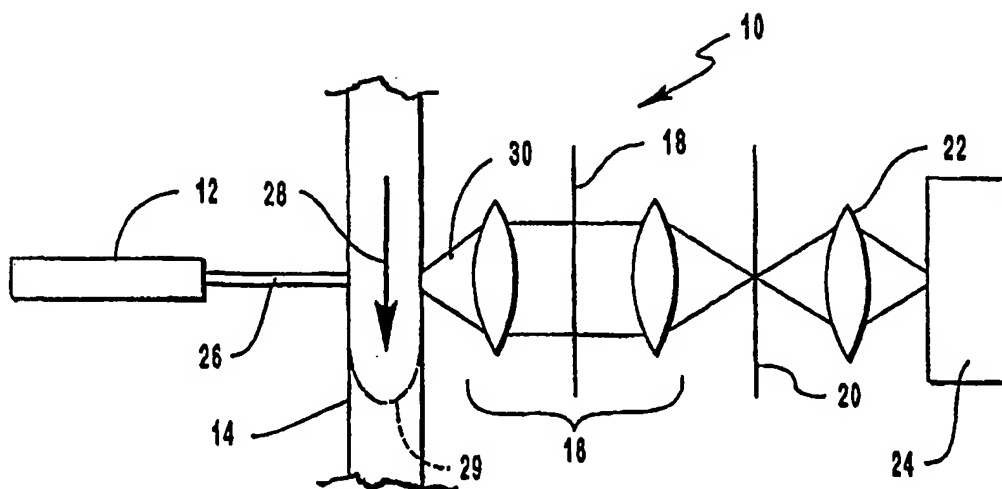
(57) Abstract

A flow cytometer (14) for detecting target particles such as microorganisms including biological cells and viruses as well as molecular species. The flow cytometer includes a detection system (10) involving a CCD (24) having a time delay integration capability to thereby increase the signal from the target particle (33) and decrease the noise detected by the CCD. Calibration particles (48) can be included in the sample stream of the flow cytometer (28) for coordinating the readout of the CCD with the rate of flow of the sample stream to improve the detection capability of the CCD. Statistical analysis techniques can also be used to determine the rate of flow of target particles in the sample stream to thereby coordinate the readout rate of the CCD with the rate of flow of the target particle.

IMPROVED FLOW CYTOMETRY APPARATUS AND METHOD

1   Background

2   Field of the Invention

3   This invention relates to flow cytometry and, more

4   particularly, to an improved flow cytometry apparatus and method

5   involving a novel time delayed integration of a charge coupled

6   device in the detection apparatus for enhancing the signal-to-noise

7   ratio.

8

9   The Prior Art

10   Cytometry is the measurement of the physical and/or chemical

11   characteristics of biological cells. Since the technology of my

12   novel discovery as disclosed and claimed herein can be adapted to

13   other systems such as viruses and molecules as well as biological

14   cells, I have adopted the term "cytometry" to refer to the

15   detection of all of these species which, for the sake of

16   convenience are referred to herein as "target particle(s)." Flow

17   cytometry, as the name implies, involves the delivery of a flowing

18   stream containing a sample having target particles therein to the

19   detection region of a flow cytometer. The target particles in the

20   detection region are irradiated using a laser to create an

21   illumination phenomena by the target particles. The appropriate

22   optics and detection electronics measure the light absorption;

23   scattering; fluorescence; and/or spectral properties of the target

1   particles in the sample, or alternatively, a fluorescent label

2   affixed to the target particles. The laser is typically a gas

3   laser (such as an Argon or a Helium Neon Laser) or even a diode

4   laser. In the case of fluorescence, each target particle produces

5   a burst of fluorescence photons as it passes through the

6   illumination region. Differentiation of the fluorescence from the

7   illumination or excitation light can be accomplished with a filter

8   or a combination of filters. Detection of the fluorescence is

9   accomplished using a photomultiplier tube or a photodiode. Another

10   technique relies on light scattering of photons in the illumination

11   beam by the target particle. The target particle is identified by

12   its light scattering as a function of the angle of scattering which

13   is a function of its size and shape as well as the wavelength of

14   the scattered photons.

15   The successful detection and identification of a single target

16   particle depends upon several factors. First, the laser power must

17   be sufficient to generate a large enough number of fluorescence

18   (or, alternatively, scattering) photons during the brief passage of

19   the target particle through the irradiation region. Specifically,

20   it is essential that a sufficient number of photons are generated

21   so that the fluorescent burst from the target particle can be

22   reliably differentiated from random fluctuations in the number of

23   background photons. Second, it is important to minimize these

24   unwanted background photons. These unwanted background photons

25   arise from scattering or from fluorescence emitted by the carrier

26   liquid of the sample or impurities in the liquid, as well as from

1   the apparatus itself, such as the walls of the capillary through

2   which the flow stream passes.

3   Flow cytometry inherently creates competition between a

4   desirable high flow rate for a reasonable sample throughput in

5   order to provide rapid detection of target particles and high

6   detectivity.   High detectivity is predicated upon having a

7   fluorescing (or scattering) target particle in the detection beam

8   long enough to provide a high signal-to-noise detection along with

9   an optical design that will minimize background photons from

10 unwanted scattering and unwanted fluorescence, both of which can

11 dominate system noise.   Optimally, a flow cytometer will have a

12 small excitation beam, a high flow rate, and detection optics and

13 associated electronics that allow for the collection of sufficient

14 photons from the target particle to provide high signal-to-noise

15 detection.

16   Various flow cytometry devices are described in available

17 references. See, for example Flow Cytometry: First Principles,

18 A.L. Givan (1992).   Other references include Sage et al. (U.S.

19 Patent No. 4,660,971); Wu et al. (U.S. Patent No. 4,661,913);

20 Yamamoto et al. (U.S. Patent No. 4,786,165); Ohki et al. (U.S.

21 Patent No. 5,007,732); and Hirako (U.S. Patent No. 5,135,302).

22 Each of these references also contain references to other relevant

23 publications.

24   As discussed previously, the competing requirements imposed on

25 flow cytometry create an inherent conflict between high sample

26 throughput and high detectivity that has proven most difficult to

3

1   overcome resulting in limitations in applications for flow

2   cytometry. In view of the foregoing, it would be an advancement in

3   the art to provide improvements in flow cytometry. It would be

4   another advancement in the art to provide a charge coupled device

5   (CCD) as the detector with the image from the target particle being

6   shifted across the CCD at the same rate that the CCD charge is

7   being shifted across the CCD. It would be an even further

8   advancement in the art to provide a flow cytometer with a time

9   delayed integration (TDI) system to significantly alter the signal-

10 to-noise ratio in favor of detection. Such a novel apparatus and

11 method is disclosed and claimed herein.

12

13   Brief Summary and Objects of the Invention

14   This invention involves the detection of target particles such

15 as microorganisms, molecules, and the like, using a CCD to provide

16 TDI of the photons from the target particle in the sample stream of

17 the flow cytometer. TDI provides a surprisingly high signal-to-

18 noise ratio of the detection photons by lowering the contribution

19 of photons from unwanted background photons with respect to the

20 photons emitted by the target particle and by lowering the

21 contribution of CCD readout noise. This high signal-to-noise ratio

22 is accomplished by effectively narrowing the field of view thereby

23 reducing the contribution by the background illumination. CCD

24 spectrophotometry can also be used to detect spectra from the

25 target particle to measure the properties of the target particle as

26 well as being able to differentiate between the scattering and

4

1   fluorescence photons from the target particle.   Further, TDI of

2   flow cytometry using a CCD accommodates simultaneous detection and

3   analysis of multiple target particles.

4   It is, therefore, a primary object of this invention to

5   provide improvements in detection of target particles using flow

6   cytometry.

7   Another object of this invention is to provide improvements in

8   the method of identifying target particles using flow cytometry.

9   Another object of this invention is to provide a flow

10   cytometry analysis of target particles with TDI.

11   Another object of this invention is to use TDI to enhance the

12   signal to noise ratio of a flow cytometry apparatus.

13   Another object of this invention is to use TDI in combination

14   with a flow cytometry apparatus to provide for the simultaneous

15   detection and analysis of multiple target particles in the sample

16   stream.

17   Another object of this invention is to coordinate the rate of

18   movement of the image across the CCD during TDI of the CCD with the

19   rate of flow of the target particles in the sample flow.

20   Another object of this invention is to provide for the facile

21   correlation between the scattering and fluorescence signals from

22   the target particles.

23   These and other objects and features of the present invention

24   will become more readily apparent from the following description in

25   which preferred and other embodiments of the invention have been

1   set forth in conjunction with the accompanying drawing and appended

2   claims.

3

4   Brief Description of the Drawing

5   Figure 1 is a schematic of a first embodiment of an imaging

6   TDI/CCD flow cytometry apparatus of this invention;

7   Figure 2 is an enlarged, schematic illustration of two target

8   particles in the core flow of the TDI/CCD flow cytometer of Figure

9   1 as they pass through the excitation zone of a laser beam;

10   Figure 3 is an enlarged schematic illustration of the face of

11   a CCD illustrating the charge shift on the CCD at the same velocity

12   as the flow velocity of the target particles;

13   Figure 4 is a schematic of a second embodiment of a TDI/CCD

14   flow cytometry apparatus of this invention for detecting two or

15   more target particles having different emission spectra;

16   Figure 5 is an enlarged, schematic illustration of two target

17   particles having different emission spectra passing through the

18   flow zone of the TDI/CCD flow cytometer of Figure 4; and

19   Figure 6 is an enlarged schematic illustration of the face of

20   a CCD illustrating the charge shift on the CCD from the two target

21   particles of Figure 5.

22

23   Detailed Description of the Preferred Embodiment

24   The invention is best understood from the following

25   description with reference to the drawing wherein like parts are

1   designated by like numerals throughout and taken in conjunction

2   with the appended claims.

3

4   General Discussion

5   A CCD is a solid state device designed to act as an

6   "artificial retina." A dense array of light receptors or pixels

7   are formed over the surface of the CCD. Light striking a pixel

8   creates an electronic charge proportional the intensity or number

9   of photons in the light that strike the pixel during exposure.

10 Conventionally, a CCD image of an object is created by opening a

11 shutter and exposing the CCD to the object. The shutter is then

12 closed and the CCD is read by transferring each charge, row by row,

13 into a serial transport register. After each row transfer, the

14 individual pixels are transferred, one at a time, through an on-

15 chip output amplifier and digitized. The readout and digitization

16 are generally performed as rapidly as possible, restrained only by

17 the existing electronic noise conditions.

18   The coupling of TDI techniques to a CCD generally requires

19 each row to be shifted more slowly in order to synchronize the

20 pixel shift rate with the rate at which the image (of target

21 particles in the flow stream of the flow cytometer) moves across

22 the CCD. The flow cytometry CCD is not shuttered as described

23 above but is read out on a continuous basis. Image smearing is

24 avoided by moving the image across the CCD at the same rate that

25 the CCD charge is being shifted across the CCD. Accordingly, the

26 charge imposed on each pixel that is read out will have dwelt for

7

1   an equal period of time on each pixel in one column of the CCD.

2   This means that all of the image pixels read from a single column

3   of the CCD will have been detected with nearly equal quantum

4   efficiency, the mean quantum efficiency of the CCD column.

5   When used for improving the signal-to-noise ratio for imaging,

6   TDI will permit one to obtain a signal-to-noise many times higher

7   than when using a single detector such as a conventional

8   photomultiplier tube.   This means that stray photons from the

9   background are significantly minimized when compared to the overall

10   signal from the target particle.   For example, one could use a

11   conventional photomultiplier tube to detect emission from a 1 μm

12   bacteria in a column of liquid with an illuminated length of 200 μm

13   and a 50 μm square cross section. Alternatively, one could use a

14   CCD with 9 μm square pixels. If a bacterium is contained in a

15   single pixel the background contribution would be reduced by over

16   a factor of more than 100. Clearly, of course, these dimensions

17   and numerical specifications are representative and are provided

18   herein for illustrative purposes only.

19   The importance of this invention is more clearly understood

20   when one considers the fact that the primary source of noise in a

21   flow cytometer is the high level of laser emission scattered by the

22   carrier fluid, particles in the sample flow, and the components of

23   the flow cytometer itself. A notch filter at the laser line

24   frequency can reduce the intensity of this background scattering by

25   a factor of about 10^{-5}, but even then the fluorescence from unbound

26   dye molecules (in an immunofluorescence flow cytometer, for

1   example) can readily overwhelm bacterial fluorescence in a single

2   detector flow cytometer.   In conventional flow cytometry this

3   problem is addressed by illuminating and collecting light from only

4   a very small region of the flow.   On the other hand, TDI achieves

5   the same result without the need to restrict the illuminated

6   volume.   A much larger illuminated volume can be used, resulting in

7   a longer residence time for each target particle in the laser beam

8   and a corresponding increased number of collected detection

9   photons.

10   TDI also allows one to image a small volume of sample flow

11   surrounding an individual target particle without knowing exactly

12   where the target particle resides inside the volume.   When the

13   emission of a laser-illuminated flow cell is imaged onto a CCD with

14   the direction and rate of flow aligned with the column direction of

15   the CCD chip, the CCD may be read out at a rate such that the

16   fluorescence emission from a single target particle always

17   accumulates in a single moving charge packet on the CCD chip.

18   Ideally, emitted light from a single target particle will reside in

19   a single pixel.

20   In the best case, emitted light from the target particle will

21   reside in a single pixel region on the CCD.   Accordingly, the

22   benefit of TDI/CCD imaging over a single pixel detector is that the

23   contribution of background photons from scattered light and

24   fluorescence from unbound dye molecules can be decreased by several

25   orders of magnitude, depending on the diameter of the sample

1   stream, the CCD pixel size, and the magnification capability of the

2   system.

3   Additionally, TDI has benefits over traditional CCD imaging,

4   where multiple short-exposure frames might be used to track a

5   target particle moving through an illuminated flow cell. The CCD

6   and its associated electronics contribute a quantity of noise,

7   called the readout noise, each time a pixel is read from the

8   device. In TDI mode a single pixel, optimally, will follow a

9   single target particle. When the pixel is digitized it will

10 contain one contribution of readout noise. For illustration we

11 assume a readout noise of 100 electrons rms for a single pixel. If

12 non-TDI imaging is used and 100 short-exposure images are used to

13 follow a target particle through the flow and said images are

14 combined by statistical means, the combined contribution from

15 readout noise is the square root of 100 x 10 electrons rms, or 100

16 electrons rms. Also, TDI is less computationally intensive than

17 using multiple short-exposure images.

18   As is apparent from the foregoing, one of the primary

19 requirements for using TDI with CCD flow cytometry is the need to

20 accurately synchronize the TDI with the flow rate in the flow

21 cytometer. A differential between the flow rate and the TDI will

22 result in streaks appearing in the CCD "images." That is, the

23 emitted light from a target particle will extend over many pixels

24 along the column direction of the CCD. The flow rate can be

25 coordinated with the TDI rate using calibration particles in the

26 sample flow. Calibration particles can also be introduced into the

1　flow during the actual experiment as long as the calibration

2　particles have a different emission spectrum than the target

3　particle being detected.　Alternatively, sample flow through a

4　capillary will exhibit a parabolic-shaped velocity profile as a

5　result of drag imposed on the liquid stream by the surrounding

6　walls of the capillary which means that one can utilize

7　conventional statistical techniques to coordinate the TDI of the

8　CCD with the flow rate in order to minimize streaks in the CCD

9　images.

10　　　The simultaneous detection of two or more different species

11　requires that each species has a unique fluorescing wavelength

12　and/or scattering phase function.　Different emission wavelengths

13　can occur due to differences in the intrinsic fluorescence (e.g.

14　autofluorescence or bioluminescence) of the target particles or by

15　tagging them with chemical labels that have differing emission

16　wavelengths.　　Tagging then allows the use of a TDI/CCD

17　spectrophotometer as a detector that can discriminate between

18　individual target particles and in real time.　This means that the

19　individual spectra will be associated with rows of charge packets

20　on the CCD rather than having individual spots (associated with

21　single charge packets on the CCD) shifted down the CCD in the TDI

22　imaging mode.

23　　　Further, since a CCD can be binned, i.e., a rectangular

24　grouping or "bin" of pixels can be read out as a single superpixel

25　with a single contribution of readout noise thereby meaning that

26　the spectral resolution of the CCD can be changed to meet the

1   problem encountered, while minimizing readout noise.   Binning also

2   allows for faster CCD readout, with the readout speed being

3   approximately proportional to the binning factor.   For example, if

4   the CCD is binned by a factor of ten along the row dimension the

5   CCD can be read out approximately ten times faster.   This means

6   that for a single-species detection, a 768 x 512 CCD can be read

7   out as a 768 x 1 device thereby providing for an increase in

8   readout speed of approximately 500.

9

10   Flow Cytometry Flow Rate Considerations

11   Conventionally, the core diameter or cross sectional area of

12   the irradiation zone of the flow cytometer determines the volume of

13   the background solution illuminated by the laser and, therefore,

14   viewed by the detection system. The rate of solution flow through

15   this irradiation zone determines the length of time the target

16   particle is being irradiated by the laser beam. This is referred

17   to as the transit time.   Transit time is important because the

18   residual, unbound fluorescent tag in the observed volume of

19   solution contributes to the number of background photons collected

20   by the detection system. Accordingly, a large number of background

21   photons can reduce the signal-to-noise ratio to such a degree as to

22   render detection of the target particle impossible.   This means

23   that the core diameter and the transit time jointly determine the

24   signal-to-noise ratio for the detection of the target particle.

25   The critical parameter that affects the transit time is the

26   flow rate of the solution through the irradiation zone.   This

12

1   parameter is typically designated as the core volumetric flow rate,

2   q, in milliliters per second, ml/s, and is related to the cross

3   sectional area of the irradiation zone, A, in square centimeters,

4   cm^2, along with the flow velocity, v, in centimeters per second,

5   cm/s. Accordingly the cross sectional area, A, can be expressed as

6   A = q/v. If the irradiation zone is circular then its diameter, d,

7   in micrometers, can be calculated using the equation $d = 1.13 \times 10^{-4}$

8    $(q/v)^{\frac{1}{2}}$.

9   As discussed earlier, the time required to read one line of

10   the CCD chip determines the flow rate because the TDI measurement

11   requires the sample flow to be synchronized as closely as possible

12   with the charge transfer on the CCD chip. One low-cost CCD camera

13   that has TDI and binning implemented in software uses a CCD chip

14   with 9 μm square pixels and can be read out at a rate corresponding

15   to a maximum flow rate of 25 cm/s (assuming an image magnification

16   of one). Consideration of the simultaneous needs for spectral

17   dispersion (i.e., approximately 50 spectral elements) and lateral

18   binning on the CCD chip suggests an optimal core diameter of 50 μm

19   (for this particular example). Thus, the core volumetric flow rate

20   for these conditions is calculated to be 5×10^{-4} ml/s. This is a

21   key quantity because it determines the total analysis time. For

22   example, if the sample has a volume of one ml, the total analysis

23   time is predicted to be 2,000 seconds.

24   However, I anticipate that my novel TDI/CCD flow cytometer

25   will provide several improvements that will significantly decrease

26   the analysis time:

13

1   1.   Image demagnification

2   Since the TDI approach provides an improvement in the

3   signal-to-noise ratio of about 100 times over traditional flow

4   cytometry, image demagnification can be used to lengthen the

5   time that a target particle will be detected on a given pixel.

6   Assuming a demagnification of 10, the maximum flow rate of

7   25 cm/s from our discussion, above, becomes 250 cm/s. This

8   then decreases the analysis time for a fixed flow volume by a

9   factor of 10. While this approach also degrades the signal-

10 to-noise ratio it is practical due to the improved signal-to-

11 noise ratio achieved through my novel TDI approach.

12   2.   Increased core diameter

13   If the image is demagnified by a factor of 10, then the

14 core diameter can be increased to about 90 μm (for a CCD chip

15 with 9 μm pixels). Compared with a 50 μm core diameter, this

16 increases the sample volumetric flow rate by a factor of 3.2.

17 Accordingly, the foregoing analysis time is decreased from

18 2,000s to 625s.

19   3.   Faster CCD readout

20   A CCD camera that has read rates that are a factor of 10,

21 or more, faster than the nominal read rate of 160 kHz can

22 decrease the analysis time by an additional factor of 10, or

23 more.

24   4.   Decreased spectral resolution

25   A two-color scheme for simultaneous detection of two

26 species is possible. For example, using a spectral resolution

1 of 50 pixels (i.e., 512 lateral pixels binned to 50), would

2 provide adequate spectral dispersion to resolve the two

3 emission bands. The CCD can be binned to match the number of

4 different types of target particles, e.g., using only four

5 spectral elements, one for each emission band and two for the

6 background signal. This change in spectral resolution by a

7 factor of 10 changes the read time by a factor of about 10

8 which decreases the analysis time accordingly.

9

10 Clearly, of course, these four changes would not provide an

11 aggregate decrease in the analysis time of 3,200 times. In

12 particular, these changes either decrease the transit time of the

13 target particle or increase the background noise which degrades the

14 signal-to-noise ratio. However, one does have the latitude to

15 increase the analysis speed by at least an order of magnitude and

16 still have an order of magnitude increase in the signal-to-noise

17 ratio over a conventional flow cytometer. Accordingly, I presented

18 the above discussion to illustrate the point that the projected

19 sampling time can be reduced by at least an order of magnitude.

20 Importantly, although I have focused this discussion of the

21 use of TDI for the detection of target particles such as

22 microorganisms using fluorescent labels, this novel discovery is

23 not limited to microorganisms or to fluorescent measurements. For

24 example, this same technique can be applied to the detection of

25 single molecules through fluorescence or absorption spectroscopy in

26 a flow cytometer. This is possible because TDI significantly

15

1   enhances the signal-to-noise ratio of the detector by decreasing

2   the contribution of the background component from the surrounding

3   medium.   Examples of such molecular analysis are the presence of

4   explosive molecules or groundwater contaminant molecules, e.g.

5   benzene, trichloroethylene, etc.

6

7   Detailed Description

8   Referring now to Figure 1, a first preferred embodiment of the

9   novel TDI/CCD flow cytometer of this invention is shown generally

10   at 10 and includes a laser 12, a flow zone 14 having a sample flow

11   28 passing therethrough, reimaging optics 16 having a notch filter

12   18 therein, an aperture 20, a focus lens 22 and a CCD 24. Laser 12

13   emits a laser beam 26 through flow zone 14 to illuminate a first

14   target particle 32 and a second target particle 33 (Figure 2)

15   therein and to cause the same to fluoresce as fluorescence 30.

16   Fluorescence 30 is optically processed by reimaging optics 16 and

17   notch filter 18 and focused onto a field-of view aperture 20.

18   Focus lens 22 focuses the resultant fluorescence 30 onto CCD 24

19   (Figure 3) as will be discussed more fully hereinafter.

20   Sample flow 28 passes through flow zone 14 and exhibits a

21   typical, parabolic-like flow velocity profile 29 as shown

22   schematically herein by the dashed line in flow zone 14.   Flow

23   velocity profile 29 is created as a result of the surface drag

24   phenomena imparted to the liquid of sample flow 28 by the wall

25   surface of flow zone 14. This is an inherent feature of any liquid

26   stream flowing through a conduit and can be readily eliminated by

1   the conventional technique of enclosing sample flow 28 in a sheath

2   of moving air or liquid (not shown) to thereby create what is.

3   commonly referred to as a "sheath flow."

4   Referring now also to Figure 2, flow zone 14 with an

5   irradiation zone 40 shown therein is shown greatly enlarged for

6   ease of illustration. For example, laser beam 26 is shown as

7   a 200 x 50 μm laser beam illuminating flow zone 14 which is shown

8   as 50μm diameter sample steam having a cylindrical cross section.

9   A first target particle 32 is shown in sample flow 28 toward the

10   lower end of irradiation zone 40 while a second target particle 33

11   is shown entering the upper end of irradiation zone 40. A

12   calibration particle 48 is also included in sample flow 28 for the

13   purpose of allowing the operator (not shown) to suitably calibrate

14   TDI/CCD flow cytometer 10 as will be discussed more fully

15   hereinafter. Calibration particle 48 emits a calibration photon 49

16   when illuminated by laser beam 26.

17   Referring now also to Figure 3, a greatly enlarged, schematic

18   of a portion of CCD 24 is shown. Two shaded areas are shown as

19   first pixel 42 and second pixel 43 which represent fluorescence 30

20   from first target particle 32 and second target particle 33,

21   respectively. First movement arrow 44 on first pixel 42 represents

22   the movement or transfer of first pixel 42 across the face of CCD

23   24. Correspondingly, second movement arrow 45 on second pixel 43

24   represents the movement or transfer of second pixel 43 across the

25   face of CCD 24. Importantly, the rate of movement of first

26   movement arrow 44 and second movement arrow. must be nearly

1   identical to that of sample flow 28 as imaged onto CCD 24 in order

2   to minimize streaking of the digitized image obtain d by CCD 24.

3   The image of calibration particle 48 is not illustrated on CCD 24

4   for purposes of simplicity although it would be essentially

5   identical to that of either first pixel 42 or second pixel 43.

6   Referring to all of Figures 1-3, sample flow 28 passes through

7   flow zone 14 carrying target first particle 32 and second target

8   particle 33 therein along with an occasional calibration particle

9   48. Laser 12 illuminates first target particle 32, second target

10 particle 33, and calibration particle 48 with laser beam 26 as they

11 each pass through irradiation zone 40 thereby causing first target

12 particle 32, second target particle 33, and calibration particle 48

13 to fluoresce due to intrinsic fluorescence (e.g. autofluorescence

14 or bioluminescence) or due to being tagged with chemical labels

15 that have characteristic emission wavelengths. Alternatively,

16 first target particle 32, second target particle 33, and

17 calibration particle 48 can cause characteristic scattering of

18 laser beam 26. In any event, each of first target particle 32,

19 second target particle 33, and calibration particle 48 emit

20 specific illumination which is referred to herein as fluorescence

21 30 for sake of simplicity or fluorescence 49 in the case of

22 calibration particle 48. Fluorescence 30 along with fluorescence

23 49 is optically processed in reimaging optics 16 containing a notch

24 filter 18 therein. Notch filter 18 provides a filtering function

25 by filtering out a significant portion of any background scattering

26 caused by laser beam 26 generating scattering 50 from the carrier

1   fluid of sample flow 28 and even from the sidewalls of flow zone

2   14, itself.   Scattering 50 is shown as having a plurality of

3   scattering rays 50a-50c which, in the absence of notch filter 18,

4   would be picked up by reimaging optics 16 and directed to CCD 24.

5   As it is presently constructed, notch filter 18 strongly attenuates

6   light at the wavelength of laser beam 26 while permitting light of

7   the wavelengths of fluorescence 30 and fluorescence 49 to pass.

8   This is an important feature in flow cytometry since scattering

9   rays 50 are noise which could significantly overwhelm the signal

10   from first target particle 32 and second target particle 33 as well

11   as from calibration particle 48.

12   Fluorescence 30 is directed through aperture 20 by reimaging

13   optics 16 and focused by focus lens 22 onto CCD 24.   In CCD 24,

14   fluorescence 30 from first target particle 32 is recorded as first

15   pixel 42 while fluorescence 30 from second target particle 33 is

16   recorded as second pixel 43.   First target particle 32 traverses

17   flow zone 14 in sample flow 28 while first pixel 42 traverses the

18   face of CCD 24 at (ideally) an identical rate of travel (assuming

19   an image magnification of unity) as shown by first movement arrow

20   44.   Correspondingly, second pixel 43 representing second target

21   particle 33 moves across the face of CCD 24 at the same rate of

22   travel as second target particle 33 in sample flow 28 and as shown

23   by second movement arrow 45.

24   As set forth previously, sample flow 28 (in a flow zone 14

25   versus a sheath flow (not shown)) has a flow velocity profile 29.

26   As such, the distance of each of first target particle 32, second

19

1   target particle 33, and calibration particle 48 from the axis of

2   sample flow 28 will be represented by a differential in the

3   velocity of flow of the respective particle.   Accordingly, the

4   rates of movement of first movement arrow 44 and second movement

5   arrow 45 will be different as a result of the spatial separation of

6   the respective target particle, first target particle 32 and second

7   target particle 33, from the side wall of flow zone 14.

8   Accordingly, this differential in the rates of movement of first

9   target particle 32 and second target particle 33 will result in

10 streaking of one or both of their images as recorded on CCD 24 as

11 first pixel 42 and second pixel 43, respectively. However, by the

12 application of relatively straight-forward statistical analysis

13 techniques one can readily compensate for this flow phenomena

14 caused by flow velocity profile 29. Further, one can use this same

15 statistical analysis tool to provide a method for determining the

16 rate of flow for a majority of first target particle 32 and second

17 target particle 33 in flow stream 28 to thereby effectively control

18 the rate of transfer of first pixel 42 and second pixel 43,

19 respectively, across the face of CCD 24. This technique would be

20 an effective way to eliminate the requirement for the use of

21 calibration particle 48. Streaking of first pixel 42 or second

22 pixel 43 is a relatively minor problem since a substantial portion

23 of the flow of sample flow 28 is at or near the axis of flow as can

24 be seen from flow velocity profile 29. Thus, while flow at the

25 wall will have zero velocity, one can set the charge transfer rate

26 on CCD 24 by measuring the streak length of target particles near

20

1   the axis of the flow. The enhancement of the signal-to-noise of

2   fluorescence 30 through TDI results in a corresponding diminution

3   of the effect of the signal from scattering ray 50b, particularly

4   since scattering rays 50 tend to be distributed throughout sample

5   flow 28 whereas fluorescence 30 is localized which results in a

6   very low amount of signal from scattering ray 50b being recorded by

7   CCD 24 in the same pixel, first pixel 42 or second pixel 43, as

8   representative of the respective target particle, first target

9   particle 32 or second target particle 33.

10   Referring now to Figure 4, a second embodiment of my novel

11   TDI/CCD flow cytometer is shown generally at 60 and includes a

12   laser 62, a flow zone 64, reimaging optics 66 with a notch filter

13   68 therein, a slit 70, a collimator 72, a grating 74, a focusing

14   lens 76, and a CCD 78. Laser 62 directs a laser beam 80 through

15   flow zone 64 causing a first target particle 82 and a second target

16   particle 83 (Figure 5) to fluoresce or otherwise emit fluorescence

17   photons 84.   Fluorescence photons 84 are optically processed

18   through imaging optics 66 and notch filter 68 and are focused

19   through aperture slit 70 and then collimated by collimator 72 and

20   directed against grating 74. Grating 74 is designed to separate

21   different wavelengths of fluorescence photons 84 prior to directing

22   the same to CCD 78 as will be discussed more fully hereinafter.

23   Sample flow 88 also includes a flow velocity profile 89

24   similar to flow velocity profile 29 (Figure 1) so that the

25   discussion thereof would be identical to that previously discussed,

21

1   particularly with respect to using statistical analysis of sample

2   flow 88 as a means for calibrating the readout of CCD 78.

3   Referring now to Figures 5 and 6, a portion of flow zone 64 is

4   shown greatly enlarged and as having two different types of target

5   particles therein, first target particle 82 and second target

6   particle 83. Flow zone 64 is illuminated by laser beam 80 which

7   causes first target particle 82 to emit a first fluorescence 96 and

8   second target particle 83 to emit a second fluorescence 97 as well

9   as a scattering fluorescence 90a-90c. In this particular

10 embodiment of TDI/CCD flow cytometer 60, a first band 94 across

11 flow zone 64 and containing first target particle 82 is read by CCD

12 78 as a first spectrum 102 while a second band 95 across flow zone

13 64 and containing second target particle 83 is read by CCD 78 as a

14 second spectrum 103.

15   Sample flow 88 also includes a calibration particle 98 which

16 emits a calibration photon 99 when illuminated by laser beam 80.

17 Calibration particle 98 allows the operator (not shown) of TDI/CCD

18 flow cytometer 60 to calibrate the operation of CCD 78 to the flow

19 rate of sample flow 88 as will be discussed therein before with

20 respect to target particle 48 (Figure 2).

21   First spectrum 102 moves across the face of CCD 78 at a rate

22 illustrated schematically by first movement arrow 104 while second

23 spectrum 103 moves across the face of CCD 78 at a rate illustrated

24 schematically by second movement arrow 105. The rate of travel of

25 first movement arrow 104 and second movement arrow 105 are each

26 carefully coordinated with the rate of travel of sample flow 88 as

1   determined through the use of calibration particle 98. This is

2   readily accomplished by detecting calibration photon 99 on CCD 78

3   and coordinating its rate of movement across CCD with that of

4   sample flow 88.

5   First spectrum 102 is shown having a particular cross hatch

6   while second spectrum 103 is shown with a different cross hatch to

7   illustrate that the spectra, first spectrum 102 and second spectrum

8   103, have emissions at different wavelengths since first target

9   particle 82 is a different species from that of second target

10   particle 83. Further, the location of first target particle 82 can

11   be at any position inside first target zone 92 while the location

12   of second target particle 83 can be at any position inside second

13   target zone 93 since both first target zone 92 and second target

14   zone 93 are read out as first fluorescence 96 and second

15   fluorescence 97, respectively. First fluorescence 96 is recorded

16   by CCD 78 as first spectrum 102 while second fluorescence 97 is

17   recorded by CCD as second spectrum 103.

18   The present invention may be embodied in other specific forms

19   without departing from its spirit or essential characteristics.

20   The described embodiments are to be considered in all respects only

21   as illustrative and not restrictive. The scope of the invention

22   is, therefore, indicated by the appended claims rather than by the

23   foregoing description. All changes which come within the meaning

24   and range of equivalency of the claims are to be embraced within

25   their scope.

23

1   What is claimed and desired to be secured by United States

2   Letters Patent is:

1

2   1.   An   apparatus   for   identifying   a   target   particle

3   comprising:

4   a sample stream containing said target particle;

5   a flow zone for said sample stream;

6   illumination means for illuminating said flow zone

7   thereby illuminating said target particle in said sample

8   stream as said target particle passes through said flow zone;

9   detection means for detecting illumination from said

10   target particle; and

11   time delay integration means for integrating said

12   illumination over a preselected interval of time.

1   2.   The apparatus defined in claim 1 wherein said sample

2   stream is contained in a flow cytometer.

1   3.   The   apparatus   defined   in   claim   1   wherein   said

2   illumination means comprises a laser means.

1   4.   The apparatus defined in claim 3 wherein said laser means

2   comprises a laser selected from the group consisting of a gas laser

3   and a solid state laser.

1   5.   The apparatus defined in claim 1 wherein said sample
2   stream includes a tag for tagging said target particle, said tag
3   causing said target particle to emit said illumination for
4   identifying said target particle.

1   6.   The apparatus defined in claim 1 wherein said sample
2   stream includes a calibration particle for calibrating the rate of
3   flow of said sample stream with said time delay integration means.

1   7.   The apparatus defined in claim 1 wherein said time delay
2   integration means includes a CCD means for increasing registration
3   of said illumination from said target particle while decreasing
4   registration of scattered light.

1   8.   The apparatus defined in claim 7 wherein said time delay
2   integration means includes statistical analysis means for analyzing
3   a flow profile of said sample stream for controlling said time
4   delay integration means with the rate of flow of said sample
5   stream.

1   9.   The apparatus defined in claim 1 wherein said sample
2   stream includes at least a first target particle and a second
3   target particle, said first target particle emitting a first
4   illumination having a first wave length and said second target
5   particle emitting a second illumination having a second wavelength.

1   10.   An apparatus for enhanc d detection of a target particle

2   in a sample stream comprising:

3   a flow zone for said sample steam;

4   radiation means for radiating said target particle in

5   said sample steam as said sample stream passes through said

6   flow zone, said radiation means causing an illumination from

7   said target particle;

8   optical means for optically processing illumination from

9   said target particle;

10   CCD means for registering said illumination optically

11 processed by said optical means; and

12   integration means for integrating said illumination

13 registered by said CCD means.

1   11.   The apparatus defined in claim 10 wherein said flow zone

2   is included in a flow cytometer.

1   12.   The apparatus defined in claim 10 wherein said sample

2   stream includes a calibration particle for calibrating said TDI of

3   said CCD.

1   13.   The apparatus defined in claim 10 wherein said

2   integration means includes statistical analysis means for analyzing

3   the velocity of said target particles in said sample stream for

4   calibrating said TDI of said CCD relative to the flow rate of said

5   sample stream.

1   14. The apparatus defined in claim 10 wherein said sample

2   stream includes at least a first target particle having a first

3   spectrum and a second target particle having a second spectrum,

4   said CCD means registering said first spectrum and said second

5   spectrum.

1   15. A method for detecting a target particle in a sample

2   stream comprising the steps of:

3   preparing a flow zone for said sample stream;

4   flowing said sample stream with said target particle

5   through said flow zone;

6   illuminating said target particle with a first

7   illumination from an illumination source, said target particle

8   emitting a second illumination;

9   detecting said second illumination from said target

10   particle with a detector to produce a signal; and

11   integrating said signal over a period of time thereby

12   increasing the signal-to-noise ratio of said signal from said

13   second illumination from said target particle.

1   16. The method defined in claim 15 wherein said detecting

2   step comprises recording said illumination with a CCD.

.1   17. The method defined in claim 16 wherein said recording

.2   step includes analyzing said sample str am using statistical

.3   analysis means for coordinating said integrating step with the rate

.4   of flow of said sample stream.

1   18. The method defined in claim 15 wherein said flowing step

.2   includes incorporating a calibration particle in said sample stream

.3   thereby providing calibration for said integrating step.

1   19. The method defined in claim 15 wherein said flowing step

2   include placing more than one tag in said sample stream, said tags

3   thereby providing more than one of said second illumination.

.1   20. The method defined in claim 15 wherein said preparing

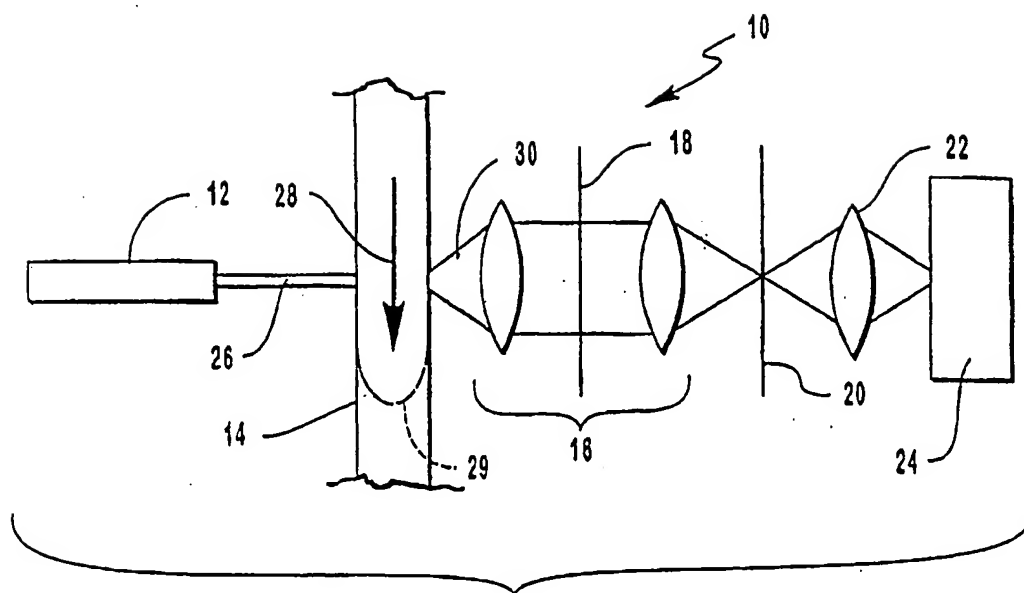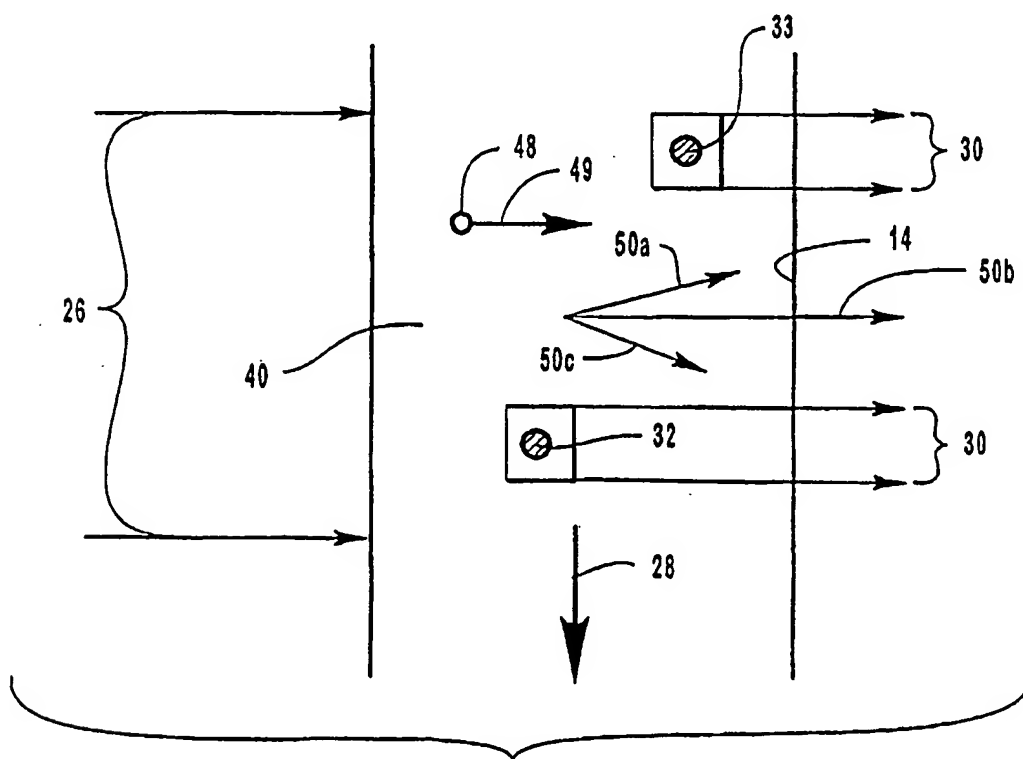.2   step includes forming said flow zone in a flow cytometer.
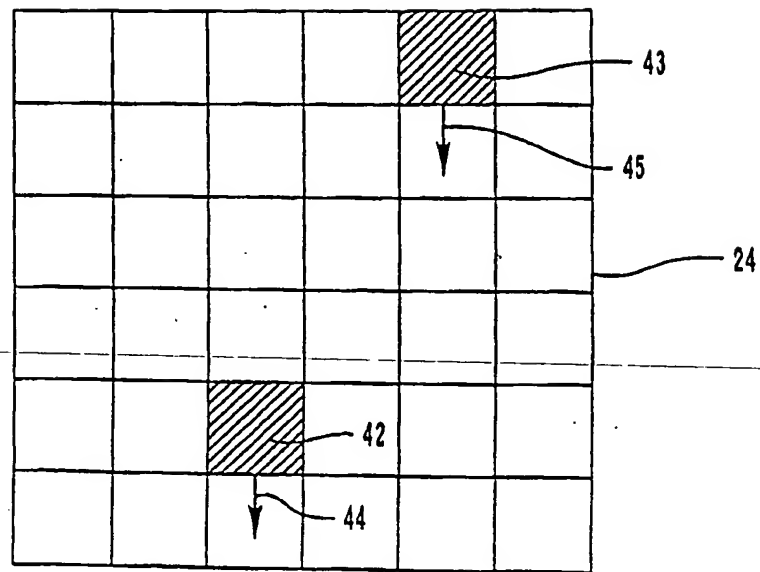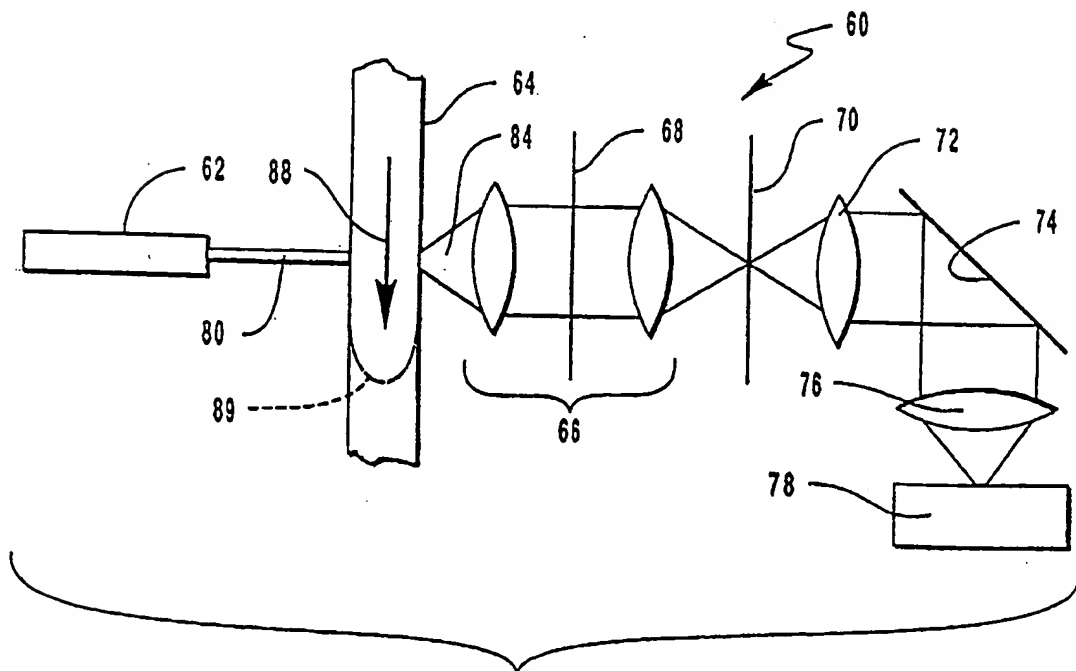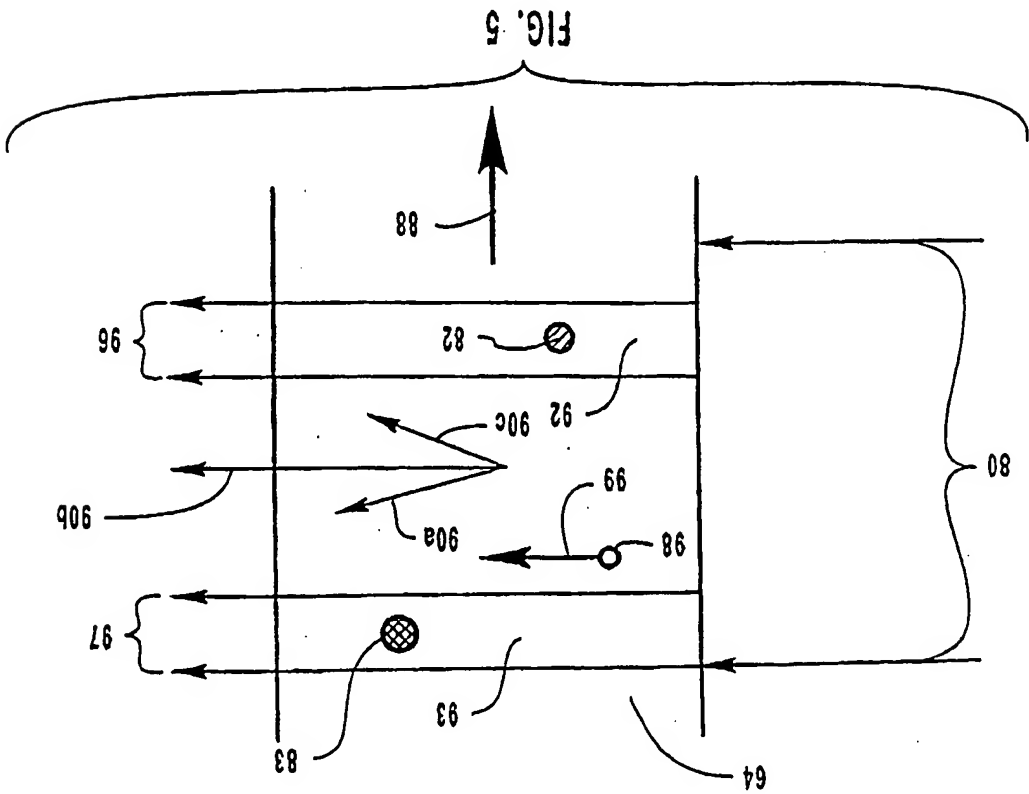
FIG. 1

FIG. 2

FIG. 3
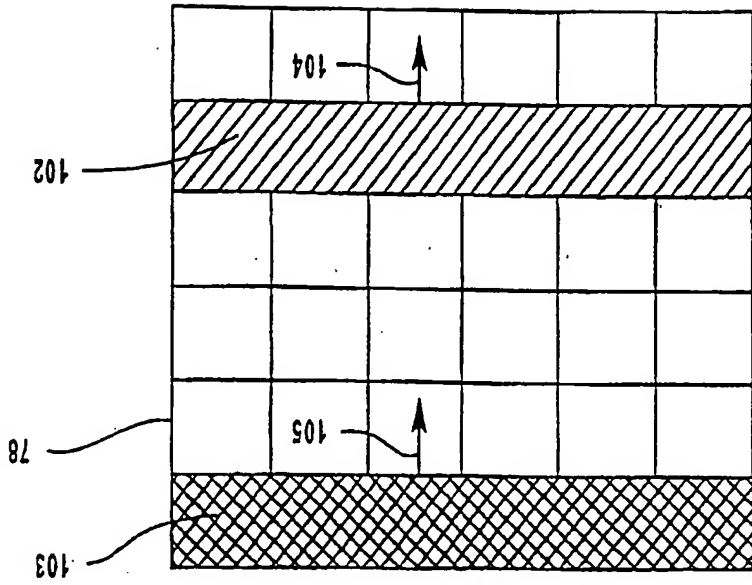

FIG. 4

3 / 3

FIG. 5

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/00573

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 15/02
US CL. : 356/335

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/335-343, 73, 317, 39, 72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

US PTO APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,444,527 A (KOSAKA) 22 August 1995 (22-08-1995), entire document.	1-20
Y	US 5,674,743 A (ULMER) 07 October 1997 (07-10-1997), entire document.	1-20

☐ Further documents are listed in the continuation of Box C.	☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 MARCH 2000	17 APR 2000

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Frank G. Font Telephone No. (703) 308-4881

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